**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY**

**WASHINGTON, D.C. 20460**

**OFFICE OF**
**PREVENTION, PESTICIDES**
**AND TOXIC SUBSTANCES**

TXR No:0050518

**Date:** February 28, 2002

**MEMORANDUM**

**SUBJECT:** ***ENDOSULFAN: RE-EVALUATION of Toxicology Endpoint Selection for Dermal and Inhalation Risk Assessments and 3X Safety for Bioaccumulation-*** Report of the Hazard Identification Assessment Review Committee.

**FROM:** Robert F. Fricke, Ph.D.
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**THROUGH:** Elizabeth Doyle, Ph.D., Chair
and
Jess Rowland, Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

**TO:** Diana Locke., Risk Assessor
Reregistration Branch II
Health Effects Division (7509C)

**PC Code: 079401**

On February 7, 2002, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) re-evaluated the toxicology database for Endosulfan to select toxicity endpoints for short, intermediate and long-term occupational/residential dermal and inhalation exposure risk assessments, as well as the 3X uncertainty factor applied to intermediate-term dermal and inhalation exposure. The toxicology endpoints selected for acute and chronic dietary risk assessments remained the same. THIS DOCUMENT SUPERSEDES THE PREVIOUS HIARC DOCUMENT DATED JANUARY 31, 2000. (TXR No. 014024)

Committee Members in Attendance

Members present were: Ayaad Asaad, Bill Burnam, Pam Hurley, David Nixon, Jess Rowland, Yung Yang, Jonathan Chen, Paula Deschamp, John Liccione, Virginia Fornillo (Exec. Secretary)

Member(s) in absentia: Elizabeth Doyle

Data evaluation prepared by: Robert Fricke, Reregistration Branch II

Also in attendance were: Diana Locke, Jennifer Tyler, Jon Punzi, Renee Sandvig, Sherrie Kinard Pauline Wagner, Stacey Milan (SRRD)

Data and Report Presentation: _____

Robert F. Fricke, Ph.D.
Toxicologist

# 1 INTRODUCTION

On September 1, 1998 the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) evaluated the toxicology data base of endosulfan, established acute and chronic Reference Doses (RfDs), evaluated the carcinogenic and mutagenic potential and selected the toxicological endpoints for occupational as well as residential exposure risk assessments. On January 31, 2000, the HIARC re-evaluated the toxicology database and selected endpoints for short-, intermediate, and long-term dermal and inhalation exposure. Additionally, the HIARC added a 3X uncertainty factor of the data from the 21-day dermal study for intermediate and long-term dermal exposure risk assessment. The HIARC also addressed the potential sensitivity of infants and children from exposure to Endosulfan as required by the Food Quality Protection Act (FQPA) of 1996. On February 7, 2002**,** the HIARC re-evaluated the toxicology end points selected, as well as the additional 3X factor based on re-evaluation of the 21-day dermal study and submission of a toxicokinetic study.

Endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 6, 9, 9a - hexahydro - 6, 9 - methano - 2, 4, 3-benzodioxathiepin-3-oxide) is a chlorinated hydrocarbon. Technical grade endosulfan is a mixture of two geometric isomers of a synthetic chlorinated cyclodiene, the alpha and beta isomers. These isomers are in concentration of 70% and 30%, respectively. Endosulfan was introduced in 1956 as an experimental broad spectrum pesticide. Endosulfan is an insecticide and acaricide of the cyclodiene subgroup which acts as a poison to a wide variety of insects and mites on contact. Endosulfan is used primarily on a wide variety of food crops including tea, coffee, fruits, and vegetables, as well as on rice, cereals, maize, sorghum, or other grains. Formulations of endosulfan include emulsifiable concentrate, wettable powder, ultra-low volume (ULV) liquid. Applications of the emulsifiable concentrate and wettable powder formulations are generally foliar, using aircraft or ground equipment.

# 2 HAZARD IDENTIFICATION

## 2.1 Acute Reference Dose (RfD)

Study Selected: Acute Neurotoxicity Study in Rats OPPTS 870.6200

MRID No.: 44403101

Executive Summary: In a neurotoxicity study, male and female Wistar rats (10/sex/dose) were fasted overnight and then orally gavaged once with endosulfan (98.6%) suspended in 2% starch mucilage at a constant volume of 10 ml/kg body weights. Two separate control groups of 10 rats/sex were used in the study. One control group was assigned to males, dosed at 25, 50 and 100 mg/kg and females dosed at 3, 6 and 12 mg/kg. The other control group was assigned to males, dosed at 6.25 and 12.5 mg/kg and females at 0.75 and 1.5 mg/kg. Rats were observed for 15 days and survivors were sacrificed on week

three. The treated groups were dosed at levels of 0 (vehicle), 6.25, 12.5, 25, 50 and 100 mg/kg for the males and 0 (vehicle), 0.75, 1.5, 6 and 12 mg/kg for the females. The study animals were evaluated for neuro-behavioral effects (FOB and motor activity) on day 7 prior to dosing, and days 1 (within 8 hours after dosing), 8 and 15 of post-dosing. Neuropathological examinations were carried out at terminal sacrifice (on week 3) on ten rats/sex of controls and four 100 mg/kg male rats and five 12 mg/kg female rats. Six males dosed at 100 mg/kg and one female dosed at 12 mg/kg died or were found dead at the day of dose administration.

Treatment-related clinical signs were noted within 8 hours after dosing on day one (peak-time of effects) in males at 50 and 100 mg/kg and females dosed at 6 and 12 mg/kg. These symptoms were not observed after day 2 in all survivors. Clinical signs noted included tonoclonic convulsions, decreased spontaneous activities, stilted gait, stupor, prone position, squatting posture, straddled hindlimbs, bristle coat, palpebral fissure narrowing, and irregular respiration and panting in males dosed at 50 and 100 mg/kg and females dosed at 6 and 12 mg/kg. In addition, increased incidences of the following signs; stilted gait, squatting posture, irregular respiration and decreased spontaneous activities in males dosed at 25 mg/kg; increased incidences of squatting posture, straddled hindlimbs, decreased spontaneous activities, bristle coat, irregular respiration and panting were also noted in females dosed at 3 mg/kg/day. Animals with "drawn in flanks" were only noted in females dosed at 3, 6, 12 mg/kg. Tremors were noted in three and four females dosed at 6 mg/kg and 12 mg/kg, respectively and in four males dosed at 50 mg/kg. Salivation was noted in one male dosed at 100 mg/kg, and in one female each dosed at 6 and 12 mg/kg. According to the study, the clinical effects observed were due to interaction of endosulfan with the brain gamma amino-butyric acid (GABA) receptors. No compound-related effects on motor activity were noted for rats that survived. No treatment-related effects were seen on: the rearing frequency, fore and hind-limb grip strength, and on landing foot-spread; body weight and food consumption; organ weight; gross pathology; or histo(neuro) pathology. The NOAEL was 12.5 mg/kg for males and 1.5 mg/kg for females. The LOAEL was 25 mg/kg for males based increased incidences of stilted gait, squatting posture, and irregular respiration, as well as decreased spontaneous activity. The LOAEL was 3 mg/kg for females, based on an increased incidence of stilted gait, squatting posture, straddled hindlimbs, irregular respirations, panting and bristled coat and decreased spontaneous activity.

Dose and Endpoint for Risk Assessment: NOAEL= 1.5 mg/kg based on increased incidences of convulsions seen within 8 hours after dosing in females at 3 mg/kg.

Comments about Study/Endpoint: The database included a lower NOAEL (maternal) of 0.7 mg/kg/day in the rabbit developmental toxicity study (MRID 00094837), based on salivation, convulsions, rapid breathing, and hyperactivity seen at 1.8 mg/kg/day. The Committee, however, decided not to use this NOAEL for this (acute) scenario because the clinical signs in the dams were seen on day 10 of gestation (i.e., after 4 treatments) whereas in the acute neurotoxicity study, convulsions were seen 8 hours after a single oral dose, thus making this endpoint more appropriate for this risk assessment.

Uncertainty Factor (UF): 100 (10x for inter-species variation and 10x for intra-species

extrapolation).

$$\textbf{Acute RfD} = \frac{\textbf{1.5 mg / kg (NOAEL)}}{\textbf{100 (UF)}} = \textbf{0.015 mg / kg}$$

## 2.2 Chronic RfD

Study Selected: Combined Chronic/Carcinogenicity Study in Rats 870.4300 (§83-5)

MRID No.: 41099502

Executive Summary: In a combined chronic/oncogenicity study (MRID 41099502), groups of 50 Sprague-Dawley rats/sex/group were fed ( in the diet) with technical endosulfan (97.1% purity) at 0, 3.0, 7.5, 15.0, and 75.0 ppm ( . 0, 0.1, 0.3, 0.6, and 2.9 mg/kg/day for males and 0, 0.1, 0.4, 0.7, and 3.8 mg/kg/day for females) for 104 weeks. A satellite group of twenty rats/sex was dosed in a similar fashion and was used for hematology and clinical chemistry evaluations. No treatment-related effects on clinical signs, mortality, food consumption and urinalysis were observed. Mean body weights of the males and females dosed at 75.0 ppm were statistically significantly decreased ($p<0.01$; 17.6%) as compared to their respective controls. Grossly, enlarged kidneys were noted in females in the satellite group dosed at 75.0 ppm (8/20 *versu*s 2/20 in the controls).

No treatment-related changes were noted in the clinical chemistry and hematology parameters evaluated. Marginal decreases of leukocyte (at week 26) and lymphocyte counts (at weeks 26 and 52) were noted in the males dosed at 75.0 ppm. At week 13, RBC counts and MCV values were decreased in all treated females as compared to the controls. Since dose related trends were not evident and since no changes were noted at other intervals, these changes were not judged to be related to treatment. Increased incidences of blood vessel aneurysms (18/70 *versus* 10/70 in controls) and enlarged lumbar lymph nodes (19/70 *versus* 14/70 in controls) were noted in the male rats dosed at 75.0 ppm as compared to the controls. Increased incidences of enlarged kidneys were seen in females dosed at 75 ppm (30/70 *versus 2*1/70 in controls) as compared to the controls. Other organ weights were not affected by dosing. Although slightly decreased testes weights were observed in males dosed at 15 and 75 ppm, these changes were not considered toxicologically significant.

Histopathologically, increased incidences of blood vessel aneurysms (18/70 *versus* 9/70 in controls) were noted in male rats dosed at 75.0 ppm. Also, a significant increased incidence of marked progressive glomerulonephrosis in the kidneys was seen in male (30/70 *versus* 20/70 in controls) and in female (8/70 *versus* 1/70 in controls) rats dosed at 75.0 ppm. The incidence of the glomerulonephrosis in the kidneys in the high-dose males (43%) was higher than that observed in the historical controls data (reported at 19.7%). This data was re-evaluated because of some concerns expressed by one member of the

RfD/RfC Work Group (Memorandum: L Taylor to G. Ghali, March 19, 1993). It was stated in this memo that the increase in the severity of progressive glomerulo-nephrosis in rats of both sexes at the high-dose level was regarded as an adverse effect and that the spontaneously occurring renal disease was exacerbated by exposure to the test material. No treatment-related neoplastic lesions were evident in this study. A slight increased incidences of pituitary adenoma in males and females dosed at 75 ppm and fibroma/ adenoma of the mammary glands females dosed at 75 ppm were not judged to be related to treatment, because dose-related trends were not evident The doses used in this study appear to be adequate to test the carcinogenic potential of the test compound, as evidence by the compound-related systemic effects noted above.

Based on the results of this study, the systemic NOAEL is 15.0 ppm (0.6 and 0.7 mg/kg/day for males and females, respectively) and the systemic LOAEL is 75.0 ppm (2.9 and 3.8 mg/kg /day for males and females, respectively) based on decreased body weight gain in male and female rats, enlarged kidneys and increased incidences of marked progressive glomerulonephrosis and blood vessel aneurysms in males.

Dose and Endpoint for Risk Assessment: NOAEL= 0.6 mg/kg/day. The LOAEL= 2.9 mg/kg/day, based on reduced body weight gain, enlarged kidneys and increased incidences of marked progressive glomerulonephrosis in males and females, and blood vessel aneurysms in kidneys of male rats.

Comments about Study/Endpoint: The RfD/Peer Review considered the chronic toxicity study in dogs (MRID41099501) with a NOAEL of 0.65 mg/kg/day to be a co-critical study. In this dog study, the LOAEL of 1.75 mg/kg/day was based on decreased body weight gain in males and increased incidences of neurologic findings in males and females (loss or weakening of placing and righting reactions, tonic contractions of abdominal muscle and masticatory muscles a few hours after feeding. The HIARC concurred with the conclusions reached by the RfD/Peer Review Committee with regard to the study, dose and endpoint used in establishing the RfD.

Uncertainty Factor (UF): 100 (10x for inter-species variation and 10x for intra-species extrapolation).

$$\textbf{Chronic RfD} = \frac{\textbf{0.6 mg / kg / day (NOAEL)}}{\textbf{100 (UF)}} = \textbf{0.006 mg / kg / day}$$

## 2.3 Occupational/Residential Exposure

### 2.3.1 Incidental Oral Exposure

There are no residential uses at the present time. Therefore, toxicology endpoints

were not selected for incidental oral exposure risk assessments.

### 2.3.2 Dermal Absorption

Two dermal absorption studies were available.

Study Selected: Dermal Absorption studies in rats OPPTS 870.7600 §85-3

MRID No.: 40223601 and 41048504

Executive Summary: In a dermal absorption study (MRID40223601), three groups of 24 male Crl: CD(SD) Br rats/group were dosed topically with radio labeled endosulfan dosing suspension (94.6% purity) at nominal doses of 0.1, 1, and 10 mg/kg and exposed for 0.5, 1, 2, 4, 10 and 24 hours. The application site was shaved and then cleaned with acetone to remove surface fats and oils and to extract some lipoid from the skin, 5 hours before dosing. Then the compound was applied onto the application site (3.7 cm in diameter = 10.8 $cm^2$). After exposure, the application sites were washed with 5 ml of mild soap solution and three 5 ml portions of water for further analysis. The animals were sacrificed and the application sites were washed with 5 ml of 1% liquid ivory soap and three 5 ml portions of water. The skin wash, filter paper, rubber ring, application site and adjacent skin, untreated skin, liver, kidney, brain and fat were analyzed for the presence of radio labeled compound. The percent doses absorbed over a 24-hour period were 2.2-21.6, 0.32-21.52, and 0.08-8.38 for the 0.1, 1, and 10 mg/kg dose groups, respectively. The percentages of endosulfan absorbed at 1, 10 and 24 hours intervals, were 1.8, 7.6 and 21.6% for rats dosed at 0.1 mg/kg, 0.57, 5.77 and 21.52%, for rats dosed at 1.0 mg/kg, and 0.29, 3.86, and 8.38% for rats dosed at 10 mg/kg. The percent doses remaining in/on the skin after soap and water washes over a 24-hour period were 62.1-56.5, 78.1-57.7, and 80.2-66.7 for the 0.1, 1, and 10 mg/kg dose groups, respectively. This data showed that significant portions of the dose remained on the skin of male rats following soap and water wash was performed. At 24-hour interval, the data showed that endosulfan bioaccumulate in the body of the rats.

In another dermal absorption study (MRID 41048504), three groups of 16 female Crl:CD(SD)BR rats/group were applied topically with radiolabeled endosulfan (purity 94.6%) at nominal doses of 0.1, 1, and 10 mg/kg (1.9, 21.9, and 231.4 mg/cm2) to determine the fate of the residue that was left in/on the skin following 10 hours of exposure. The application sites were shaved one day before dosing. Thirty minutes before dosing the sites were cleaned with acetone to remove surface fats and oils and to extract some lipoid from the skin. A rubber ring was glued on the shaved application site, then the compound was applied onto an application site within the rubber ring, and afterwards a filter paper was cemented

on the rubber ring. Ten hours after dosing, the application sites were washed with 1% liquid Ivory soap and rinsed with water. The skin wash, filter paper, rubber ring, application site and adjacent skin, untreated skin, liver, kidney, brain, fat, muscle, blood, urine, feces, and carcass were analyzed for the presence of radio labeled compound. The radioactive labeled endosulfan presence was analyzed in four live rats/group at 24, 48, 72 and 168 hours after dosing. The percent doses absorbed at 24 hours were 22.1, 16.1 and 3.8% and at 168 hours were 44.8, 46.4 and 20.3% for the 0.1, 1, and 10 mg/kg dose groups, respectively. The percentages of the doses remaining on/in the skins at 168 hours were 41.4, 56.2 and 72.8% for the 0.1, 1, and 10 mg/kg dose groups, respectively. The data showed that endosulfan bioaccumulate in the body of the rats.

Dermal Absorption Factor: The HIARC selected the dermal absorption factors of 45 % (rounded from 44.8%) at 168 hours post exposure.

Comments about Dermal Absorption: The Committee selected the dermal absorption rate based on the following weight-of-evidence considerations: 1) at 24 hours, the percent absorption was comparable between males (21.6%) and females (22.1%); 2) in female rats, even after washing at 10 hours, the percent absorption increased with time, the final measurement was 44.8% at 168 hours; 3) the concern that the test material continued to be absorbed even after washing at 10 hours; 4) substantial dermal absorption is demonstrated in the 21-day dermal toxicity study with a NOAEL of 3 mg/kg/day and systemic toxicity (increased mortality, and increased liver abnormalities) evident at 9 mg/kg/day (LOAEL). In addition, this dermal absorption factor is supported by comparing the results of the oral and dermal studies in the same species. The ratio of the oral LOAEL of 6 mg/kg/day in the developmental toxicity study in rabbits and the dermal LOAEL of 9 mg/kg/day in the 21-day dermal toxicity study in rabbit with the same endpoint (increased mortality) indicate a dermal absorption rate of 67% [(6 ÷9] X 100 = 67%) as compared to the amount absorbed orally.

### 2.3.3 Dermal Occupational/Residential Exposure

#### 2.3.3.1 Short-Term Dermal - (1-30 days)

Study Selected: 21-Day Dermal Toxicity Study in Rats OPPTS 870.7600 (§82-2 )

MRID No. 00146841, 00147744

Executive Summary Study 1: In a 21-day dermal toxicity study, endosulfan (97.2% w/w) was applied onto the skin of five groups of six male and female Wistar rats at doses of 0, 1, 3, 9, and 27 mg/kg/day and onto six males only at 81

mg/kg/day, for 21 applications (5 days a week) over 30 days. Five of the six (83%) high-dose (27 mg/kg/day) females died on days 2 and 6 of the study. Three of the six (50%) high-dose (81 mg/kg/day) males died on days 2 and 3 of study (females were not tested at this dose). Two of the three 81 mg/kg/day males that died showed tonoclonic convulsions, increased salivation and respiration. Although no deaths occurred in males dosed at 27 mg/kg/day, 2 of the 6 (33%) males dosed at 9 mg/kg/day died on days 5 and 8; these deaths were not considered to be treatment-related due to possible preexisting health problems. Increased incidences of mortality in males dosed at 81 mg/kg/day and females dosed at 27 mg/kg/day appear to be a compound-related effect. No changes of clinical chemistry and hematology parameters can be attributed to treatment. Changes that occurred were small and they are not judged to be dose-related. Females dosed at 9 mg/kg/day showed significantly increased absolute and relative spleen and absolute adrenal weights, as compared to controls. Significant dermal irritation was not produced by the test compound. Dermal irritation for all groups was very slight at all evaluation intervals. It appears that dermal irritation was more persistent in females at 3 and 9 mg/kg/day dose groups, as evidenced by greater dermal irritation scores (2-3 times) than that of controls. There was no difference between the average scores of the treated males as compared to the controls at any dose level. Although dermal irritation scores were zero at the end of the study, and although the pathology report described that dermal effects were similar in treated and control animals, there appears to be an increase in severity or prolongation of irritation found in females dosed at 3 and 9 mg/kg/day.

**The NOAEL was established at 9 mg/kg/day in females and 27 mg/kg/day in males, based on increased mortality at LOAELs of 81 mg/kg/day in males and 27 mg/kg/day in females.**

Executive Summary Study 2: In a 21-day dermal toxicity study (MRID 00146841), endosulfan (97.2%) was applied to the skin of five groups of 11 male and 11 female Wistar rats at doses of 0, 12, 48, 96, and 192 mg/kg in males and 0, 3, 6, 12, and 48 mg/kg in females for 21 applications over 30 days. Six rats/sex/group were kept as the main treatment group for 29 days, and then sacrificed. After being treated for 29 days, the remaining five rats/sex/group were kept untreated for another 14 days before they were sacrificed.

Treatment-related signs of toxicity (pilo-erection, salivation and lacrimation) were noted in 96 mg/kg males. Non-treatment related signs of toxicity (piloerection, slight lacrimation and auto-aggression) was noted in one female (#99) dosed at 12 mg/kg dose level. Eight females dosed at 48 mg/kg showed treatment-related clinical signs (three showed hypersalivation, 2 of which died; one showed crusted eyes; one bloody crusted nose which subsequently died; one showed dacryohemorrhea, tonic convulsions, marked salivation and bloody exudate).

Two males dosed at 192 mg/kg (one each on days 6 and 9) and four females dosed at 48 mg/kg (between days 2 and 22) died after showing tonoclonic convulsions. Increased mortalities in the males dosed at 192 mg/kg (2 of 11 rats) and females dosed at 48 mg/kg (four out of 11 rats) are considered to be treatment-related. One female each of the groups dosed at 3, 6, and 12 mg/kg died on day 18 from unknown causes. The investigator speculated that these mortalities were attributed to the application technique employed and for this reason this study was immediately repeated using a modified application method at dose levels of 0, 1, 3, 9, 27 and 81 mg/kg/day (Hoechst AG Rept# A30764, p.17). Since the methods were not sufficiently described in the report, no conclusion can be made whether or not the methods used were improper. The deaths, however, did not appear to demonstrate typical Endosulfan toxicity. Skin dryness and desquamation were noted in all groups.

The body weight, food consumption, hematology, and urinalysis values were not affected by the treatment.

Significant differences in a number of clinical chemistry parameters as compared to the controls were noted in the males at day 30: both sodium and chloride (at 96 mg/kg), SGPT (at 12 mg/kg), and protein (at 192 mg/kg). Protein in the 96 mg/kg females was also statistically significantly elevated at day 30. These changes are not considered to be toxicologically significant, because they were sporadic and/or dose-related trends were not evident. On day 30 evaluation, inhibitions of serum ChE (- 33%), RBC ChE (-28%) and brain ChE (- 17%) activity was noted in the males dosed at 192 mg/kg, 48 mg/kg and 96 mg/kg, respectively. Only the depressed serum ChE activity (33%) in males dosed at 192 mg/kg as compared to the controls appeared to be treatment-related.

At day 30, statistically significant increases in relative kidney weights were noted in the males dosed at 12, 96, and 192 mg/kg; the absolute kidney weights, however, were not statistically significantly increased.

Discrete deposition of pigments in a few cells of the proximal straight tubule of the kidneys was noted; this finding is of interest, because similar pigment deposition in the proximal convoluted tubules was noted in other studies with endosulfan.

Scattered erythema and edema were also noted, but they disappeared at the end of the treatment period; these findings were not judged to be treatment-related, because dose-related trends were not noted. EndosuLfan was nonirritating at all doses tested.

**The NOAEL was established at 12 mg/kg/day in females and 96 mg/kg/day in males, based on increased mortality in males and females and increased**

**serum ChE activity inhibition in males at LOAELs of 192 mg/kg/day in males and 48 mg/kg/day in females.**

This study is classified as **acceptable/guideline**, and it satisfies the guideline requirements of a repeated dermal toxicity study in rats (82-2).

Dose and Endpoint Selected for Risk Assessment: NOAEL= 12 mg/kg/day based on mortality in females at 27 mg/kg/day (LOAEL).

Comments about Study/Endpoint: The NOAEL and LOAEL for dermal exposure were obtained from two separate 21-day dermal studies. The NOAELs and LOAELs for female rats were 9 and 27 mg/kg/day, respectively, for one study (MRID 00147744) and 12 and 48 mg/kg/day, respectively, for the other study (MRID 00146841). NOAEL is appropriate for this specific route for dermal exposure scenarios up to 30 days. Previously, the HIARC selected the NOAEL of 3 mg/kg/day based on mortality of two males on days 2 and 3 of the study at 9 mg/kg/day. A re-evaluation of the data showed that these deaths were not treatment related, but rather due to possible preexisting health conditions of the animals. This was supported by the observation of no mortality at the next higher dose of 27 mg/kg/day. Additionally, in a second dermal study, no mortality occurred even at 48 mg/kg/day. Therefore, the HIARC determined that the 12 mg/kg/day is the appropriate NOAEL for risk assessment. This dose/study/endpoint are appropriate for this duration and scenario.

### 2.3.3.2 Intermediate-Term Dermal (30 Days to 6 months)

Study Selected: 21-Day Dermal Toxicity Study in Rats OPPTS 870.7600 (§82-2 )

MRID No. 00146841, 00147744

Executive Summary: See Short-Term Exposure

Dose and Endpoint Selected for Risk Assessment: NOAEL= 12 mg/kg/day based on mortality in females at 27 mg/kg/day (LOAEL). The endpoints from two 21-day dermal toxicity studies were considered in arriving at the NOAEL (MRID 00147744) and LOAEL (MRID 00146841).

Comments about Study/Endpoint: The NOAEL and LOAEL for dermal exposure were obtained from two separate 21-day dermal studies. The NOAELs and LOAELs for female rats were 9 and 27 mg/kg/day, respectively, for one study (MRID 00147744) and 12 and 48 mg/kg/day, respectively, for the other study (MRID 00146841). **There is no evidence to indicate that long term exposure increases toxicity in target organs due to bioaccumulation, the HIARC**

**recommended that the factor of 3X uncertainty factor be removed.** This conclusion was based on the results of a toxicokinetic study (MRID 45546201) with $^{14}C$-labeled endosulfan. In this study was administered to 4 Wistar rats/sex/dose in by gavage] at a dose level of 1 mg/kg/day for up to 28 days. Interim sacrifices were carried out to evaluate the time course for accumulation of labeled residues in tissues and blood. Groups of animals were dosed daily for 28 days, followed by a treatment-free period lasting up to 5 days. During the treatment-free period, fecal and urinary excretion of labeled residues were evaluated over a four day period; blood was collected over a 120 hour interval. Pharmacokinetic analysis was performed on the blood and tissue data.

Following repeated daily dosing, the labeled tissue residue concentrations increased, reaching maximum concentrations by day 23. The highest concentrations of labeled residues were found in the kidney, which peaked at 43 mg/kg (mg endosulfan equivalents/kg tissue) in males and 32 mg/kg in females. Liver concentrations peaked at 4.2 mg/kg in males and 6.9 mg/kg in females. Five days after the last dose, kidney residue concentrations decreased to 27 mg/kg (37% decrease) in males and 25 mg/kg (22% decrease) in females, while liver concentrations decreased to 1.7 mg/kg (60% decrease) in males and 3.2 mg/kg (53% decrease) in females. The concentration of residues in other tissues were lower with peak concentrations of 0.24 to 1.2 mg/kg in males and 0.30 to 3.0 mg/kg in females. Analysis of the kidney residues showed that the major metabolite was endosulfan sulfate; no apolar residues were found.

Following 28 days of dosing, overall recovery of labeled residues , expressed as the percentage of the total administered dose, was 9.253% in males and 9.794% in females **(i.e. over 90% of the radioactivity was eliminated prior to the 28th dose).** Approximately 60 - 63% of the labeled endosulfan was eliminated in the feces, while urinary elimination accounted for approximately 12 - 14%.

Pharmacokinetic analysis of the blood residue concentrations indicated that both the Cmax (maximum concentration) and half-life were higher in the male (1.64 mg/kg and 147 hr, respectively) than in the female (0.685 mg/kg and 98 hr, respectively). The time to peak concentration, however, was shorter in males than females (6 hr vs 8 hr).

#### 2.3.3.3 Long-term (greater than 6 months)

The current use pattern for endosulfan does not indicate the potential for long-term exposure. Therefore, no dose and endpoint were not selected.

### 2.3.4 Inhalation Exposure

### 2.3.4.1 Short-Term (1-30 Days)

Study Selected: 21 Day Inhalation in the Rat OPPTS 870.3465 (§82-4)

MRID No.: 00147183

Executive Summary: In a range-finding study (MRID 41667501) two groups of 5 Wistar rats/sex were exposed, nose-only, to aerosol concentrations of endosulfan (97.2%) at 0.0024 and 0.0065 mg a.i./L for 6 hours/day, five days/week for a total of 7 exposures. Two females exposed to 0.0065 mg/L died by Day 8 of the study. Female survivors had clinical signs including, tremors, trembling, tonic-clonic convulsions and reduced corneal reflexes. Males exposed to the highest concentration were ataxic and had irregular breathing. Body weight loss were noted in males and females at both concentrations early in the study (days 3-4). Based on the results of this range finding study, the highest concentration for the 21-day subchronic study was set at 0.0020 mg a.i./L. In a 21-day inhalation toxicity study (MRID 00147183), ten male and ten female Wistar rats were exposed, nose-only, to technical endosulfan (97.2% pure) at concentrations of 0 (air), 0.0005, 0.0010, and 0.0020 mg/L air (0.097, 0.194, and 0.387 mg/kg/d)[1] for 6 hours/day, 5 days/week for a total of 21 exposures over 29 days. An additional group of 5 animals/sex/dose were held for a 4-week recovery period after receiving the test aerosol. No mortality or clinical signs of toxicity occurred during the study. Group mean body weights were similar to controls with the exception of males in the highest dosed group that had lower body weight (3-5%) from day 20 through 29. In the highest dosed males from the recovery group, the decrements in body weights were more pronounced (12-16%) from recovery days 34-60. Although neither sex had any statistically significantly body weight changes during the exposure period and the number of recovery animals for each sex was only 5, the apparent effect suggested a possible delay in its manifestation.

Erythrocyte counts in the low and mid dose males at the end of the exposure period (Day 29) were significantly elevated. No effects on erythrocyte counts were observed at the high dose, hence the changes did not demonstrate a pattern of toxicity because the toxicological significance of an increased RBC count is unknown. In addition, the test report stated that the values were apparently within the norm for the species and strain studied. Some slight effects on clinical chemistry and in hematology counts were noted but these did not demonstrate significant toxicity of the test compound. There were statistically significant

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[1] Conversion of mg/L to oral dose (mg/kg/day) = mg/L X absorption (1.0) X[Respiratory Volume (Wistar rats) for 6 hours/day] X Duration of Exposure (5days/wk)/ body weight X 7 days/week

$$= \frac{0.001 \text{ mg/L} \times 1.0 \times [8.46(\text{RV}) \times 6 \text{ hrs}) \times 5 \text{ d/wk}}{0.187 \text{ kg} \times 7 \text{ d/wk}} = 0.194 \text{ mg/kg/day}$$

decreases in leucocyte counts (20.1%) in the high-dose males, which seemed to be marginally dose related but did not indicate significant toxicity. High-dose females had increased creatinine (21%) values suggestive of kidney toxicity and were judged to be treatment related but there were no other supporting kidney toxicity in histopathology or organ weight changes.

The NOAEL was 0.0010 mg a.i./L (0.20 mg/kg/day), the LOAEL was 0.0020 mg a.i./L (0.40 mg/kg/day), based on decreased body-weight gain and decreased leukocyte counts in the males and increased creatinine values in the females.
Dose and Endpoint Selected for Risk Assessment: NOAEL = 0.0010 mg a.i./L (0.2 mg/kg/d) based on decreased body-weight gain and decreased leukocyte counts in males and increased creatinine values in females at the LOAEL of 0.0020 mg a.i./L (0.4 mg/kg/d ).

Comments about Study/Endpoint: The dose and endpoint are appropriate for 30 days, since it is based on a route-specific study of 21-days duration.

### 2.3.4.2 Intermediate-Term Inhalation (30 Days to 6 months)

Study Selected: 21-Day Inhalation in the Rat OPPTS 870.3465 (§82-4)

MRID No.: 00147183

Executive Summary: See Intermediate-Term Inhalation Exposure

Dose and Endpoint for Risk Assessment: NOAEL = 0.0010 mg a.i./L (0.2 mg/kg/d) based on decreased body-weight gain in both sexes and decreased leukocyte counts in males and increased creatinine values in females at the LOAEL of 0.0020 mg a.i./L (0.4 mg/kg/day).

Comments about the Study/Endpoint: The 21-day study is also appropriate for intermediate-term exposure scenarios because of the appropriateness of the route of exposure. The target organ (decreased body weight gain and increased creatinine in females) of concern are appropriate early markers for the effect observed in rats following long-term oral exposure (decreased body weight and kidney disease).

**Since there is no evidence to indicate that long-term exposure increases toxicity in target organs due to bioaccumulation, the HIARC recommended that the factor of 3X be removed.** This conclusion was based on the results of a toxicokinetic study (MRID 45546201) with $^{14}$C-labeled endosulfan which showed that endosulfan did not bioaccumulate.

#### 2.3.4.3 Long-term Inhalation (greater that 6 months)

The current use pattern for endosulfan does not indicate the potential for long-term exposure. Therefore, no dose and endpoint were not selected.

### 2.4 Margins of Exposure for Occupational/Residential Exposures

A MOE of 100 is adequate for short- and intermediate-term occupational dermal and inhalation exposures risk assessments. There are no residential uses at the present time.

### 2.5 Recommendation for Aggregate Exposure Risk Assessments

There are no residential uses at the present time; therefore aggregate exposure risk assessment is not required.

## 3 CLASSIFICATION OF CARCINOGENIC POTENTIAL

### 3.1 Combined Chronic Toxicity/Carcinogenicity Study in Rat 870.4300 (§83-5)

MRID No. 41099502

Executive Summary: See Chronic Dietary

Discussion of Tumor Data: There was no evidence of carcinogenicity.

Adequacy of the Dose Levels Tested: The Committee considered that the doses tested were adequate to test the carcinogenic potential of the test compound.

### 3.2 Carcinogenicity Study in Mice

MRID No. 40792401

Executive Summary: In a carcinogenicity study, groups of HOE:NMRKf (SPF71) 60 mice/sex/group were fed with technical endosulfan (97.9% purity) at 0, 2, 6 and 18 ppm (**.** 0, 0.3, 0.9 and 2.6 mg/kg/day) for 24 months. A satellite group of twenty mice/sex/group was dosed in a similar fashion and were used for hematology and clinical chemistry evaluations. No treatment-related effects were evident in clinical signs, food consumption, hematology, clinical chemistry, urinalysis, organ weights and gross and microscopic evaluations. At study termination, statistically significant increased mortality was noted in the high-dose females (only 28% survivors *versus* 45% of the controls). Increased mortality in the high-dose females is judged to be treatment-related. Mean body

weights of the males and females were comparable among all groups. A slight reduction in body weight gain in high-dose males (between weeks 13 and 26) was noted. At 12 months, the lung and ovary weights of the 18 ppm females were significantly ($p<0.05$) decreased and at 18 months, the relative liver weights of males dosed at 18 ppm and relative ovary weights in females dosed at 18 ppm were slightly but significantly decreased. At 24 months, organ weights were comparable among all groups. Decreases in organ weights noted at various intervals during the study are not judged to be related to treatment, because they are within the normal historical ranges. Histopathologically, slight increased incidences of epithelial thickening of the urinary bladder were noted in all treated males (0, 5, 8 and 12, in the control, low-, mid- and high-dose groups, respectively) and females (0, 6, 9 and 10, in the control, low-, mid- and high-dose groups, respectively). The original reviewer of this study concluded these increases were not toxicologically significant, because of the "absence of a progression to a clear proliferative change". Lymphosarcoma was found in practically all organs of male and female mice. In addition, since it was noted equally among all groups, these occurrences were judged to be spontaneous and strain-related changes. These occurrences were not considered to be treatment-related effects. The systemic NOAEL was 6 ppm (0.9 mg/kg/day) and the LOAEL was 18 ppm (2.65 mg/kg/day), based on increased incidences of mortality in females.

This study is classified as **acceptable/ guideline** and it satisfies the guideline requirements for an oncogenicity study in mice (§83.2).

Discussion of Tumor Data: There was no evidence of carcinogenicity. The lymphosarcoma observed in all organs of male and female mice of both treated and control mice at comparable incidences were judged to be spontaneous and strain-related changes, and were not considered to be treatment-related effects.

Adequacy of the Dose Levels Tested: The Committee considered that the doses used in this study were adequate to test the carcinogenic potential of endosulfan.

## 4 MUTAGENICITY

Endosulfan technical was inactive in the primary rat hepatocyte unscheduled DNA synthesis (UDS) assay (MRID00148265), and was non-mutagenic in the mouse lymphoma forward mutation assay (MRID00148266).

## 5 FQPA CONSIDERATIONS

### 5.1 Adequacy of the Database

The toxicology data base is adequate for FQPA assessment.

Acceptable prenatal toxicity studies in rats and rabbits, and a 2-generation reproduction toxicity study in rats using endosulfan were submitted to the Agency. The HIARC identified the subchronic neurotoxicity study as a data gap.

## 5.2 Neurotoxicity

In a 42-day delayed neurotoxicity study (MRID 00147181), endosulfan technical (97.2% a.i.) in corn oil was administered by oral gavage to 40 white leghorn hens at 96 mg/kg. The result of this neurotoxicity study is inconclusive; in the 9/40 animals examined at 42 days after initial and challenge (day 21) dosing, there was no evidence of progressive nerve damage in the brain, spinal cord, or peripheral nerve. No evidence of a delayed neurotoxicity or neuropathology was observed in an acute delayed neurotoxicity study using endosulfan in hens.

The acute neurotoxity study (MRID 44403101) is described in Section 2.2 , Acute Reference dose. The NOAEL was 12.5 mg/kg for males and 1.5 mg/kg for females. The LOAEL was 25 mg/kg for males based increased incidences of stilted gait, squatting posture, and irregular respiration, as well as decreased spontaneous activity. The LOAEL was 3 mg/kg for females, based on an increased incidence of stilted gait, squatting posture, straddled hindlimbs, irregular respirations, panting and bristled coat; decreased spontaneous activity was also noted.

## 5.3 Other evidence of neurotoxic effects

In a subchronic feeding study, decreased serum ChE activity was noted in female rats dosed at 360 mg/kg/day (40% at week 13) (MRID 00145668).

In a subchronic dermal toxicity study, neurological signs (tremors, Straub-tail, trismus, saltatory spasms, extension spasms and tetanoid spasms) were noted right after dosing in males dosed at 81 mg/kg and in females dosed at 18 and 36 mg/kg, with isolated incidences noted in females dosed at 12 mg/kg. These signs occurred 1 hour after dosing and disappeared 30 minutes after the onset of neurological signs. Since the number of these neurological signs was not presented in the study report, the toxicological significance of these findings cannot be evaluated with certainty. Decreased serum ChE activity was seen in female rats 80 mg/kg/day. (MRID 41048505).

In another subchronic dermal toxicity study), decreased serum ChE activity was noted in female rats dosed at 192 mg/kg/day and tonoclonic convulsions were noted in females dosed at 48 mg/kg/day (MRID 00146841, 00147744).

In a chronic toxicity feeding study in dogs, neurological effects were only noted in dogs

dosed at 30/45/60 ppm (1.75 mg/kg/day). Increased incidences of neurologic findings in males and females were characterized by loss or weakening of righting reactions, and tonic contractions of the abdominal and masticatory muscles (MRID 41099501).

In a developmental toxicity study in rats, dams dosed at 6 mg/kg exhibited tonoclonic seizures, increased salivation, and hyperactivity (MRID 43129101).

In a developmental toxicity study in rabbits (MRID 00094837), does dosed at 1.8 mg/kg showed tonoclonic convulsions, rapid breathing, increased salivation, and hyperactivity. There are no indicators for any special sensitivity to the fetuses that are evident in either the rat or the rabbit studies.

### 5.4 Developmental Toxicity

In a developmental toxicity study (MRID 43129101), endosulfan technical (97.3% a.i.) was administered by gavage to four groups of 20 pregnant female Wistar rats at dose levels of 0, 0.66, 2.00, and 6.00 mg/kg/day from days 6 through 18 of gestation. The following treatment-related, effects were noted in dams dosed at 6.00 mg/kg/day dose level: 1) the deaths of four dams and two non-pregnant females; 2) decreased body weight gain (23% of control) during the first week of dosing and concomitant food consumption decrease (68% of control); 3) tonoclonic convulsions in 16 dams, three of which showed increased salivation and one of the latter also exhibited hyperactivity. No statistically significant differences were noted in the number of corpora lutea/dam, implantations/dam, live fetuses/dam, resorptions/dam, dead fetuses/dam, pre- and post-implantation losses, litter weight, fetal body weight (combined and per sex), or fetal crown-rump length among the groups. Only one malformed fetus was found in the 6.00 mg/kg/day dose group. There was, however, an increased incidence in the number of "retarded" fetuses (fetal weights of less than 3 gms) at the 6.00 mg/kg/day dose group (8 *versus* 5 litters in controls). The original reviewer considered the increased incidences of thoracic vertebral centra fragmentation and an increased incidence of "retarded" fetuses (fetuses weighing less than 3 grams) in the 6.00 mg/kg/day dose group, to be treatment-related. No other significant fetal malformations were noted. For maternal toxicity, the NOAEL was 2.00 mg/kg/day and the LOAEL was 6.00 mg/kg/day, based on increased death, tonoclonic convulsions, increased salivation, and decreased bodyweight gains and food consumption. For developmental toxicity, the NOAEL was 2.00 mg/kg/day and the LOAEL was 6.0 mg/kg/day, based on a slight increase in the incidence of fragmented thoracic vertebral centra and a slight increase in the occurrence of "retarded" fetuses (fetuses weighing less than 3 grams). There are no indicators of any special sensitivity to the fetus in this study.

In a developmental toxicity study (MRID 00094837), endosulfan technical (97.3% a.i.) was administered by gavage to four groups of 20 pregnant female New Zealand White rabbits at dose levels of 0, 0.3, 0.7, and 1.8 mg/kg/day from days 6 through 28 of gestation. The does were sacrificed on gestation day 29. The following effects were

noted in the 1.8 mg/kg/day dams: 1) Four does dosed at 1.8 mg/kg/day died on gestation days 7, 10, 21 and 29. Three of them were due to improper oral gavage as evidenced by the presence of oil in the trachea and the lungs and one doe dosed at 1.8 mg/kg/day that died showed evidence of hemorrhagic activity. 2) Increased incidences of convulsions, rapid breathing, salivation and hyperactivity were also noted in does dosed at 1.8 mg/kg/day.3) Body weight losses were noted in does dosed at 0.7 and 1.8 mg/kg/day (-16 and -47 g/rabbit *versus* 43 g/rabbit in the controls) during days 19-29 but these values were not statistically significant. The body weight (after corrected for uterine weight) was only negative in does dosed at 1.8 mg/kg/day as compared to the controls (-17 g *versus* 5g/rabbit in the controls). No treatment-related effects on fetal deaths/resorptions, altered growth, developmental anomalies and malformations were noted. Developmental toxicity was not observed at any dose level. For maternal toxicity, the NOAEL was 0.7 mg/kg/day and the LOAEL was 1.8 mg/kg/day, based on decreased bodyweight, increased incidences of deaths, convulsions, rapid breathing, salivation and hyperactivity. For developmental toxicity, the NOAEL was greater than 1.8 mg/kg/day, the highest dose tested; a LOAEL was not established.

## 5.5 Reproductive Toxicity

In a 2-generation reproduction study (MRID 00148264), exposure of Crl:COBS CD(SD)BR rats to endosulfan (97% purity) via the diet during premating and through gestation and lactation, at dose levels of 0, 3, 15, and 75 ppm (0, 0.20, 1.00, and 4.99 mg/kg/day in males and 0, 0.24, 1.23, and 6.18 mg/kg/day in females), produced minimal maternal toxicity at the high-dose level. There were 32 rats/sex/group in the $F_0$ generation and 26 rats/sex/group in the $F_1$ generation. Mortality, food/water consumption, and body weight were not affected in either generation, but there was a decrease in body-weight gain in the $F_0$ females at the high-dose level during the first week of study (67% of control). Pregnancy rate, gestation times, the ability to rear young to weaning, and pre-coital time were comparable among the groups at both matings in both generations. $F_0$ males displayed increased heart weight at the mid- and high-dose levels and increased liver and kidney weights at the high-dose level. $F_0$ females displayed increased brain and liver weights at the high-dose level. In the $F_{1b}$ adults, the high-dose males displayed increased kidney weights compared to the controls and the females displayed increased liver weights at the mid- and high-dose levels. These organ weight changes were not considered to be toxicologically significant (see notes below regarding RfD Committee memo dated October 13, 1992). The litter size throughout both matings in both generations was not affected by dosing. In the first mating of the $F_0$ generation, there was an increase in the cumulative litter loss (8 litters) at the high-dose level.

Litter and pup weights were comparable at birth among the groups in both generations, but there was a decrease in litter weight observed during the lactation to weaning period in both matings in the $F_0$ generation, which was significant at the high-dose level in the first mating and at the mid- and high-dose levels in the second mating (dose-related). Because there was no corroborative finding of a decrease in the number of pups per litter or in pup weight, the decrease in litter weight is not considered to be treatment-related. Increased pituitary weights (high-dose female pups of 1$^{st}$ mating in $F_O$ generation) and increased uterine weights (high-dose female pups of 1$^{st}$ mating of $F_{1b}$ generation) were observed in

the offspring. There were no histopathological findings observed that could be attributed to treatment. Although there were no significant effects noted on the dams, the dose levels are considered adequate, based on the results of the range-finding study in which there was an increase in cumulative pup loss and a reduction in litter size at the 100 ppm dose level at days 24 and 28 days post weaning. For parental systemic toxicity, the NOAEL was 1.23 mg/kg/day and the LOAEL was 6.18 mg/kg/day, based on decreased body weight. For offspring toxicity, the NOAEL was 1.2 mg/kg/day and LOAEL was 6.18 mg/kg/day, based on increased pituitary and uterine weights. The offspring effects were not considered to be severe when compared to the maternal effects, since it was seen only in one generation (not consistent) and these were not the target organ for toxicity in other studies with endosulfan.

### 5.6 Open Literature Data

The ATSDR[2] reported a number of studies that assessed endosulfan's effects on the endocrine disruption system.

In a study [3] adult rats were dosed orally for 7 days; decreased testicular testosterone in conjunction with increased serum testosterone were observed suggesting sec-hormone binding globulin (SHBG) may be affected. In a subsequent study, these researchers performed another study in which rats were dosed rats orally for 15-30 days. Under the conditions of this study, and reported decreases in testicular testosterone, plasma testosterone, LH, and FSH as well as decreased steroidogenic enzyme and cytochrome P-450-dependent monooxygenase were reported. These decreases in LH may lead to decreases in the activity of steroidogenic acute regulatory protein (responsible for translocation of cholesterol to the inner mitochondria) and may therefore affect the conversion of cholesterol to testosterone.

In a competitive binding assay[4] using alligator oviduct tissue, endosulfan exposure significantly inhibited 3H-17$-estradiol binding to the estrogen receptor and progestin 3H-R5020 binding to the progesterone receptor.

Ramamoorthy et al.[5] used the yeast reporter system to discover endosulfan induced human-ER-mediated $-gal activation. Endosulfan induced $-galactosidase

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[2] Agency for Toxic Substances and Disease Registry, Toxicological Profile for Endosulfan (update) September 2000

[3] Singh A, Pandey, R.S. (1989), Gonadal toxicity of short term chronic endosulfan exposure to male rats. Indian J. Biol. 27: 341-346

[4] Vonier, P.M., Crain, D.A. McLachlan et al. (1996), Interaction of environmental chemicals with the estrogen and progesterone receptors of the American alligator, Environ Health Perspect. 104(12): 1318-1322.

[5] Ramamoorthy, K, Wang, F, Chen, I-C et al. (1997), Estrogenic activity of a dieldrin/toxaphene mixture in the mouse uteru, MCF-7 human breast cancer cells, and yeast-based estrogen receptor assays: No apparent synergism. Endocrinology 138(4): 1520-1527.

transcription/expression to about 32% of the induction seen after estradiol treatment at 0.01 µM.

In a study conducted by Sinha et al.[6] , rats were dosed orally with endosulfan for 70 days and observed exhibited deceases in sperm counts in the *cauda epididymis* and as well as decreased intratesticular spermatid counts.

And finally, Lakshmana et al.[7] showed endosulfan induces small but significant changes in the levels of noradrenaline, dopamine and serotonin in the developing rat brain and deficits in the operant learning performance suggesting possible effects on the neuroendocrine system.

### 5.7 Determination of Susceptibility

The data base for endosulfan is considered adequate for complete and there are no data gaps pertaining to developmental or reproductive toxicity. The data provided no indication of increased sensitivity of rats or rabbits to *in utero* and post-natal exposure to endosulfan. Two prenatal developmental toxicity studies, one in rats and one in rabbits, failed to show evidence of developmental toxicity in the absence of maternal toxicity. In the two-generation reproduction study in rats, effects in the offspring were observed only at or above treatment levels which resulted in evidence of parental toxicity.

### 5.8 Recommendation for a Developmental Neurotoxicity Study

As discussed earlier (Section 5.2. Neurotoxicity), treatment-related clinical sign of neurotoxicity was seen following oral exposures in rats, rabbits and dogs and via dermal exposure in rats. Changes in brain weights or histopathological lesions of the central or peripheral nervous system were not seen in the hens, acute neurotoxicity study in rats or in the other subchronic and chronic studies. The HIARC, however, placed the requirement for a developmental neurotoxicity study in rats in **reserve** status because of the datagap for a subchronic neurotoxicity study in rats.

## 6 HAZARD CHARACTERIZATION

Except for a datagap for a subchronic neurotoxicity study, the toxicology database is complete to assess the chronic toxicity, carcinogenicity, mutagenicity as well as the developmental and reproductive toxicity potential of endosulfan.

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[6] Sinha, N, Narayan, R, Saxena, DK (1997) Effect of endosulfan on the testis of growing rats. Bull. Environ Contam Toxicol 58(1): 79-86

[7] Lakshmana, MK, Raju, TR (1994). Endosulfan induces small but significant changes in the levels of noradrenaline, dopamine and serotonin in the developing rat brain and deficits in the operant learning performance. Toxicology 91(2): 139-150.

Endosulfan (6,7,8,9,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) is a chlorinated hydrocarbon. Technical grade endosulfan is a mixture of the alpha and beta isomers. These isomers are in concentration of 70% and 30%, respectively. The database for endosulfan is adequate to assess the toxicology hazard profile and is acceptable to support reregistration. The database includes the required acute toxicity studies; subchronic oral, dermal and inhalation studies; chronic studies, and developmental and reproductive toxicology studies. The mutagenic and carcinogenic potentials of endosulfan were also evaluated. In addition to these studies, metabolism, dermal absorption and pharmacokinetic studies were carried out with $^{14}C$-labeled endosulfan.

Endosulfan is a chlorinated cyclodiene pesticide, and like other members of this chemical group, the predominant toxicological effect is over stimulation of the central nervous system [by inhibiting $Ca^{2+}$, $Mg^{2+}$-ATPase and antagonizing chloride ion transport in GABA (gamma-aminobutyric acid) receptors] with little or no peripheral component. Convulsions (seizures) are the most important symptoms of endosulfan toxicity. Characteristic clinical signs following acute exposure are indicative of central nervous system (CNS) disturbances or over stimulation and include, hyperactivity, uncoordination, seizures, convulsions and death. Although these effects were not generally observed at the LOAEL, at higher doses, they were observed in the acute and subchronic toxicity studies and developmental studies in the rat and rabbit. In a chronic feeding study, dogs also exhibited central nervous system disturbances such as abnormal righting reflexes, tonic contractions, involuntary muscle movements and pronounced sensitivity to noise and light.

Endosulfan is highly acutely toxic via the oral and inhalation routes of exposure, with $LD_{50}$ and $LC_{50}$ values placing it in Toxicity Category I. By the dermal route, however, endosulfan was less toxic (Toxicity Category III). Further, endosulfan is an eye irritant in rabbits (Toxicity Category I) but is not a dermal irritant or sensitizer.

The subchronic toxicity of endosulfan was evaluated in two 13-week feeding studies in the rat and mouse, two 21-day dermal toxicity studies in the rat, and one 21-day inhalation study, also in the rat. In general, females are more sensitive to the toxic effects than males. In the 13-week feeding studies, anemia occurs (consisting of decreased hemoglobin and/or decreased mean red blood cell hemoglobin concentration) at the LOAEL and higher doses in both rats and mice. Treatment-related hematological effects (anemia), however, were not observed in any of the 21-day dermal or inhalation studies. In the dermal studies in rats increased mortality was observed at the LOAEL. In one of the dermal studies, other toxic effects at the LOAEL included increased incidence of liver abnormalities in males and females and increased absolute spleen weight in females. In the other 21-day dermal toxicity study, females had hypersalivation (CNS effect) at the LOAEL. In the 21-day inhalation toxicity study, the LOAEL was established by decreased body-weight gain and decreased leukocyte counts in the males and increased creatinine values in the females.

The chronic toxicity of endosulfan was evaluated in a combined two-year feeding/ oncogenicity study in rats, a one-year feeding study in dogs, and an oncogenicity study in mice. Chronic toxicological endpoints at the LOAEL included, in part, decreased body weight gain in male and female rats and decreased body weight in male dogs. Additional effects at the LOAEL included neurological effects in female dogs, marked progressive glomerulonephrosis (kidney toxicity) in male and female rats and blood vessel aneurysms in males rats. Endosulfan did not exhibit any

oncogenicity in rats or mice.

The developmental toxicity of endosulfan was evaluated rats and rabbits. Maternal toxicity at the LOAEL included decreased body weights in rats and rabbits and increased incidence of clinical signs in rats (tonoclonic convulsions, increased salivation, mortality) and rabbits (convulsions, rapid breathing, salivation, hyperactivity, mortality). Developmental toxicity in the rat included a slight increase in the incidence of fragmented thoracic vertebral centra and a slight increase in the occurrence of microsomic fetuses. No developmental toxicity was observed in rabbits. There are no indicators of any special sensitivity to the fetus in either the rat or rabbit study; the LOAELs for developmental toxicity were equal to or greater than the LOAELs for systemic maternal toxicity.

The reproductive toxicity of endosulfan was evaluated in a two-generation study in the rat. LOAELs for parental systemic and developmental toxicity were established at the high-dose tested. The LOAEL for parental systemic toxicity was based on decreased body weight and for developmental toxicity, increased pituitary and uterine weights. The increases in pituitary gland weights are suggestive of possible effects on hormonal metabolism and endocrine function. The increased incidence of parathyroid hyperplasia in male rats in the carcinogenicity study and several open literature publications also suggest that endosulfan has hormonal effects.

Endosulfan was evaluated in an acute neurotoxicity screening battery in the rat and an acute delayed neurotoxicity study in the hen. The LOAEL in the rat study was based on behavioral disturbances such as increased incidences of stilted gait, hunched posture, irregular respiration, and decreased spontaneous activity in males and females; females also had increased incidence of straddled hindlimbs, panting and bristled coat. The acute delayed neurotoxicity study in the hen showed no evidence of progressive nerve damage in the brain, spinal cord and peripheral nerve.

Endosulfan was not carcinogenic and did not show any mutagenic potential. There was no increase in the frequency of tumors in either the rat or mouse carcinogenicity studies. Endosulfan is classified as a Group E carcinogen (evidence of non-carcinogenicity for humans) by the Agency. The submitted mutagenicity studies have satisfied the data requirements for mutagenicity testing, and there is no concern for a mutagenic effect in somatic cells. In the *in vitro* or *in vivo* mutagenicity studies, both the mouse lymphoma forward mutation assay and the unscheduled DNA synthesis assay were negative.

Studies with radiolabeled endosulfan evaluated the metabolism in the rat and mouse, the pharmacokinetics in the rat and dermal absorption in the rat. Endosulfan was found to be rapidly metabolized into mainly water-soluble compounds and eliminated with very little absorption in the gastrointestinal tract. The primary metabolites include endosulfan sulfate, endosulfan diol, endosulfan ether, endosulfan alpha-hydroxy ether, and endosulfan lactone. The metabolites accumulated in tissues, especially in the kidney and liver. Following dietary exposure to endosulfan, a large amount of endosulfan sulfate was recovered in the liver, small intestine and visceral fat with a trace of this metabolite in the muscle.

In a special pharmacokinetic study $^{14}$C-endosulfan, at a dose of 1 mg/kg/day, was administered by gavage to rats for up to 28 days, followed by a five day treatment-free period. Labeled blood and tissue residue concentrations peaked and reached a plateau by day 23. Following 28 days of

dosing, overall recovery of labeled residues, expressed as the percentage of the total administered dose, was 9% in males and 10% in females (i.e. 90% of the radioactivity was eliminated prior to the 28th dose). The half life ($t_{1/2}$) was 147 hours in males and 98 hours in females. During the treatment-free period, tissue and blood residues decreased, showing that endosulfan does not bioaccumulate in rats.

Dermal absorption studies in male and female rats showed that endosulfan is slowly absorbed through the skin and is slowly excreted which suggests that endosulfan bioaccumulates in the body. A dermal absorption factor of 45% was used for assessment of occupational and residential exposure.

There was no evidence of increased susceptibility in rat and rabbit fetuses following *in utero* exposures in prenatal toxicity studies in rats and rabbits or increased susceptibility in the offsprings as compared to parental animal following pre/post natal exposure in the two generation reproduction study.

The open literature suggests that endosulfan is suspected to affect normal hormone metabolism and endocrine function. In studies submitted to the Agency, treatment-related effects were seen in the two-generation reproduction study in rats characterized as increases in the pituitary glands weights and as increased incidences of parathyroid hyperplasia in male rats in the carcinogenicity study.

## 7 DATA GAPS

Subchronic Neurotoxicity - Rat OPPTS 870.6200 (§82-5)
Developmental Neurotoxicity Toxicity Study - Rat (reserve) OPPTS 870.6300 (§83-6)

# 8 ACUTE TOXICITY

| Guideline | Study Type | MRID | Results | Toxicity Category |
|---|---|---|---|---|
| 870.1100 | Acute Oral | 00038307 | $LD_{50}$ = 40.38 mg/kg in **%**<br>$LD_{50}$ = 9.58 mg/kg in **&** | I |
| 870.1200 | Acute Dermal | 41183503 | $LD_{50}$ = 2000 mg/kg | III |
| 870.1300 | Acute Inhalation | 41183504 | $LC_{50}$ = 0.16-0.5 mg/L | I |
| 870.2400 | Primary Eye Irritation | 255157 | Eye irritant<br>(Residual opacity at day 13) | I |
| 870.2500 | Primary Skin Irritation | 00038309 | Non-irritant | IV |
| | | 00128649 | Slightly irritant | IV |
| 870.2600 | Dermal Sensitization | 00136994 | Not a dermal sensitizer | |

## 9 SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

| EXPOSURE SCENARIO | DOSE (mg/kg/day) | ENDPOINT | STUDY |
|---|---|---|---|
| Acute Dietary | NOAEL=1.5 | Increased incidences of convulsions seen within 8 hours after dosing in females at 3.0 mg/kg | Acute neurotoxicity-Rat |
| | UF=100 | Acute RfD = 0.015 mg/kg | |
| Chronic Dietary | NOAEL = 0.6 | Reduced body weight gain and increased incidences of marked progressive glomerulonephrosis and blood vessel aneurysms in male rats at 2.9 mg/kg. | 2-year chronic toxicity/ carcinogenicity-Rat |
| | UF=100 | Chronic RfD = 0.006 mg/kg/day | |
| Short-Term -Dermal (1 to 30 Days) | Dermal NOAEL= 12 | Increased mortality in females dosed at 27 mg/kg/day | 21-day dermal toxicity-Rat |
| Intermediate-Term-Dermal (30 Days - 6 Months) | Dermal NOAEL= 12 | Increased mortality in females dosed at 27 mg/kg/day | 21-day dermal toxicity-Rat |
| Long-Term Dermal (greater than 6 months) | The current use pattern for endosulfan does not indicate the potential for long-term exposure. Therefore, no dose and endpoint were not selected. | | |
| Short-Term Inhalation (1 to 30 Days) | Inhalation NOAEL= 0.001 mg/L (0.20 mg/kg/d) | Decreased body-weight gain and decreased leukocyte counts in males and increased creatinine values in females at 0.002 mg/L (0.40 mg/kg/d) | 21-day inhalation-Rat |
| Intermediate-Term Inhalation (30 Days- 6 Months) | Inhalation NOAEL= 0.001 mg/L (0.20 mg/kg/d) | Decreased body-weight gain and decreased leukocyte counts in males and increased creatinine values in females at 0.002 mg/L (0.40 mg/kg/d) | 21-day inhalation-Rat |
| Long-term Inhalation (greater than 6 months) | The current use pattern for endosulfan does not indicate the potential for long-term exposure. Therefore, no dose and endpoint were not selected. | | |

For occupational exposures, a MOE of 100 is adequate
Note: Residential MOEs will be determined by the FQPA Safety Factor Committee.